GOVERNMENT DEGREE COLLEGE FOR WOMEN, KARIMNAGAR, TELANGANA						
1.3.2 Percentage of students undertaking project work/field work/internship (Data for the latest completed academic year)						
Name of the department	**Nature of the work**	**Title of the project work/field work/ internships**	**Programme Name**	**Programme Code**	**List of students undertaking project work/field work/internship (Upload excel file)**	**Link**
Biotechnology	Project work	Anti Bacterial Activity of Ocimum tenuiflorum	B.Sc(Life Science)		Ayesha Fathima	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Thahaseen	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Sumera Anjum	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		M.Supriya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Sanober Tabassum	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	Polymerase chain reaction	B.Sc(Life Science)		B. Vaishnavi	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		J. Supriya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		P.Pujinitha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Ch.Jyothi	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		B.Shirisha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	Anti Bacterial Activity of Azadirachta Indica	B.Sc(Life Science)		R.Manasa	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Ch.Supraja	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		J.Sravya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf

			B.Sc(Life Science)		Syeda Madihatul Ifrah	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		B.Sonali Bindre	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	Post covid complications	B.Sc(Life Science)		Madiha Afreen	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		K.Rajeshwari	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		B.Kalyani	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		S.Soujanya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Md.Afreena	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	Study of Dairy Products-Butter and Ice cream	B.Sc(Life Science)		D.Geethanjali	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		M.Sandhya Rani	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Bindhu Reddy	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		V.Shravani	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Sara Shahewar	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	Study of Dairy Products-Cheese and Butter Milk	B.Sc(Life Science)		A.Madhumitha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Sumera Khamar	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		Syeda Habeebunnisa	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		J.Akanksha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		G.Prathyusha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
		Analysis of Various Milk-Physical and Biochemical	B.Sc(Life Science)		E.Ramya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf

Biotechnology	Project work		B.Sc(Life Science)		E.Anjali	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		A.Anjali	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		A.Sravani	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		K.Ravali	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
Biotechnology	Project work	An Overview of Synthetic Seeds	B.Sc(Life Science)		K.Vikasitha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		K.Thirumala	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		K.Shalini	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		L.Srinitha	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf
			B.Sc(Life Science)		R.Sandhya	https://gdcts.cgg.gov.in//Uploads/files/Recent_Updates/109997.pdf

A Project Report
On

POLYMERASE CHAIN REACTION
(A Detailed Study about PCR and It's Applications)

By
Bachu Vaishnavi (Ht.No. 19077164572004)
Jakkula Supriya (Ht.No. 19077164572007)
Parvathi Pujinitha (Ht.No. 19077164572012)
Chirutha Jyothi (Ht.No. 19077164572006)
Banothu Shirisha (Ht.No. 19077164572005)
(B.Sc. (Bt.B.C.) III Year Students)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN
KARIMNAGAR
(Affiliated to Satavahana University)

Polymerase chain reaction

INTRODUCTION

The **polymerase chain reaction** (**PCR**) is a method widely used to rapidly make millions to billions of copies (complete or partial) of a specific DNA sample, allowing scientists to take a very small sample of DNA and amplify it (or a part of it) to a large enough amount to study in detail. PCR was invented in 1983 by the American biochemist Kary Mullis at Cetus Corporation; Mullis and biochemist Michael Smith, who had developed other essential ways of manipulating DNA, were jointly awarded the Nobel Prize in Chemistry in 1993.

PCR is fundamental to many of the procedures used in genetic testing and research, including analysis of ancient samples of DNA and identification of infectious agents. Using PCR, copies of very small amounts of DNA sequences are exponentially amplified in a series of cycles of temperature changes. PCR is now a common and often indispensable technique used in medical laboratory research for a broad variety of applications including biomedical research and criminal forensics.

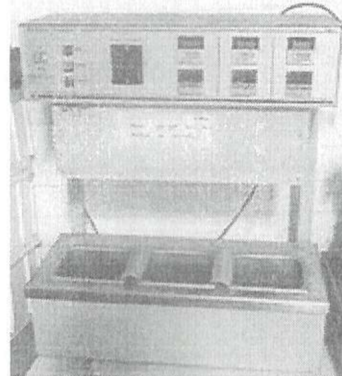
The majority of PCR methods rely on thermal cycling. Thermal cycling exposes reactants to repeated cycles of heating and cooling to permit different temperature-dependent reactions—specifically, DNA melting and enzyme-driven DNA replication. PCR employs two main reagents—primers (which are short single strand DNA fragments known as oligonucleotides that are a complementary sequence to the target DNA region) and a DNA polymerase. In the first step of PCR, the two strands of the DNA double helix are physically separated at a high temperature in a process called nucleic acid denaturation. In the second step, the temperature is lowered and the primers bind to the complementary sequences of DNA. The two DNA strands then become templates for DNA polymerase to enzymatically assemble a new DNA strand from free nucleotides, the building blocks of DNA. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the original DNA template is exponentially amplified.

Almost all PCR applications employ a heat-stable DNA polymerase, such as *Taq* polymerase, an enzyme originally isolated from the thermophilic bacterium *Thermus aquaticus*. If the polymerase used was heat-susceptible, it would denature under the high temperatures of the denaturation step. Before the use of *Taq* polymerase, DNA polymerase had to be manually added every cycle, which was a tedious and costly process.

Applications of the technique include DNA cloning for sequencing, gene cloning and manipulation, gene mutagenesis; construction of DNA-based phylogenies, or functional analysis of genes; diagnosis and monitoring of genetic disorders; amplification of ancient DNA; analysis of genetic fingerprints for DNA profiling (for example, in forensic science and parentage testing); and detection of pathogens in nucleic acid tests for the diagnosis of infectious diseases.

Principles

A thermal cycler for PCR



An older, three-temperature thermal cycler for PCR

PCR amplifies a specific region of a DNA strand (the DNA target). Most PCR methods amplify DNA fragments of between 0.1 and 10 kilo base pairs (kbp) in length, although some techniques allow for amplification of fragments up to 40 kbp. The amount of amplified product is determined by the available substrates in the reaction, which becomes limiting as the reaction progresses.

A basic PCR set-up requires several components and reagents, including:

- a *DNA template* that contains the DNA target region to amplify
- a *DNA polymerase*; an enzyme that polymerizes new DNA strands; heat-resistant *Taq* polymerase is especially common, as it is more likely to remain intact during the high-temperature DNA denaturation process
- two DNA *primers* that are complementary to the 3' (three prime) ends of each of the sense and anti-sense strands of the DNA target (DNA polymerase can only bind to and elongate from a double-stranded region of DNA; without primers, there is no double-stranded initiation site at which the polymerase can bind); specific primers that are complementary to the DNA target region are selected beforehand, and are often custom-made in a laboratory or purchased from commercial biochemical suppliers
- *deoxynucleoside triphosphates*, or dNTPs (sometimes called "deoxynucleotide triphosphates"; nucleotides containing triphosphate groups), the building blocks from which the DNA polymerase synthesizes a new DNA strand
- a *buffer solution* providing a suitable chemical environment for optimum activity and stability of the DNA polymerase

- *bivalent cations*, typically magnesium (Mg) or manganese (Mn) ions; Mg^{2+} is the most common, but Mn^{2+} can be used for PCR-mediated DNA mutagenesis, as a higher Mn^{2+} concentration increases the error rate during DNA synthesis; and *monovalent cations*, typically potassium (K) ions.

The reaction is commonly carried out in a volume of 10–200 μL in small reaction tubes (0.2–0.5 mL volumes) in a thermal cycler. The thermal cycler heats and cools the reaction tubes to achieve the temperatures required at each step of the reaction (see below). Many modern thermal cyclers make use of the Peltier effect, which permits both heating and cooling of the block holding the PCR tubes simply by reversing the electric current. Thin-walled reaction tubes permit favorable thermal conductivity to allow for rapid thermal equilibrium. Most thermal cyclers have heated lids to prevent condensation at the top of the reaction tube. Older thermal cyclers lacking a heated lid require a layer of oil on top of the reaction mixture or a ball of wax inside the tube.

Procedure

Typically, PCR consists of a series of 20–40 repeated temperature changes, called thermal cycles, with each cycle commonly consisting of two or three discrete temperature steps (see figure below). The cycling is often preceded by a single temperature step at a very high temperature (>90 °C (194 °F)), and followed by one hold at the end for final product extension or brief storage. The temperatures used and the length of time they are applied in each cycle depend on a variety of parameters, including the enzyme used for DNA synthesis, the concentration of bivalent ions and dNTPs in the reaction, and the melting temperature (T_m) of the primers. The individual steps common to most PCR methods are as follows:

- *Initialization*: This step is only required for DNA polymerases that require heat activation by hot-start PCR. It consists of heating the reaction chamber to a temperature of 94–96 °C (201–205 °F), or 98 °C (208 °F) if extremely thermostable polymerases are used, which is then held for 1–10 minutes.
- *Denaturation*: This step is the first regular cycling event and consists of heating the reaction chamber to 94–98 °C (201–208 °F) for 20–30 seconds. This causes DNA melting, or denaturation, of the double-stranded DNA template by breaking the hydrogen bonds between complementary bases, yielding two single-stranded DNA molecules.
- *Annealing*: In the next step, the reaction temperature is lowered to 50–65 °C (122–149 °F) for 20–40 seconds, allowing annealing of the primers to each of the single-stranded DNA templates. Two different primers are typically included in the reaction mixture: one for each of the two single-stranded complements containing the target region. The primers are single-stranded sequences themselves, but are much shorter than the length of the target region, complementing only very short sequences at the 3' end of each strand.

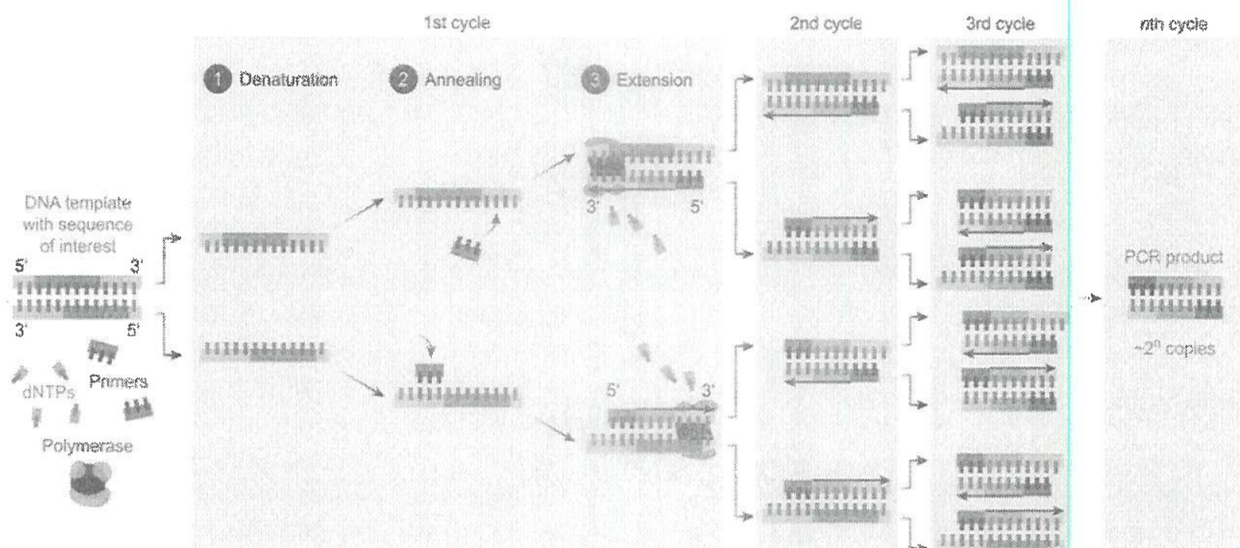
  It is critical to determine a proper temperature for the annealing step because efficiency and specificity are strongly affected by the annealing temperature. This temperature must be low enough to allow for hybridization of the primer to the strand, but high enough for the hybridization to be specific, i.e., the primer should bind *only* to a perfectly complementary part of the strand, and nowhere else. If the temperature is too low, the primer may bind imperfectly. If it is too high, the primer may not bind at all. A typical annealing temperature

is about 3–5 °C below the T_m of the primers used. Stable hydrogen bonds between complementary bases are formed only when the primer sequence very closely matches the template sequence. During this step, the polymerase binds to the primer-template hybrid and begins DNA formation.

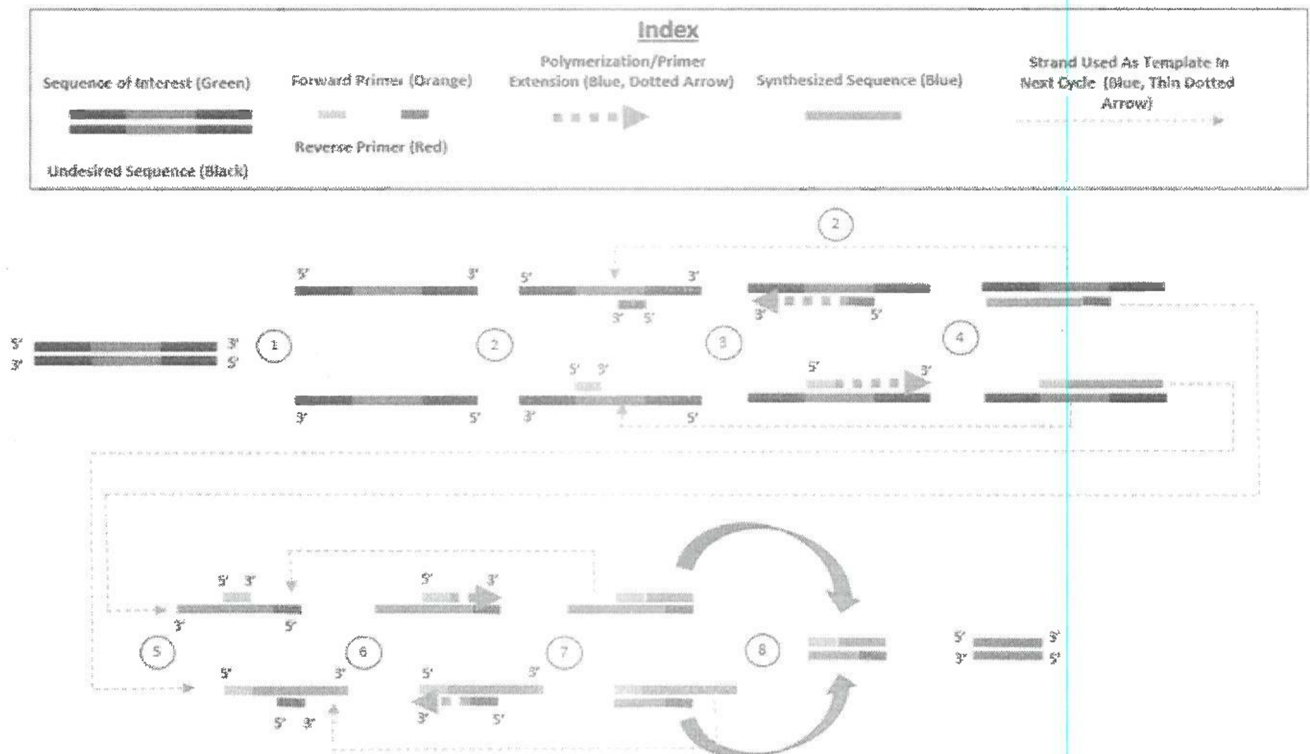
- *Extension/elongation*: The temperature at this step depends on the DNA polymerase used; the optimum activity temperature for the thermostable DNA polymerase of *Taq* polymerase is approximately 75–80 °C (167–176 °F), though a temperature of 72 °C (162 °F) is commonly used with this enzyme. In this step, the DNA polymerase synthesizes a new DNA strand complementary to the DNA template strand by adding free dNTPs from the reaction mixture that is complementary to the template in the 5'-to-3' direction, condensing the 5'-phosphate group of the dNTPs with the 3'-hydroxy group at the end of the nascent (elongating) DNA strand. The precise time required for elongation depends both on the DNA polymerase used and on the length of the DNA target region to amplify. As a rule of thumb, at their optimal temperature, most DNA polymerases polymerize a thousand bases per minute. Under optimal conditions (i.e., if there are no limitations due to limiting substrates or reagents), at each extension/elongation step, the number of DNA target sequences is doubled. With each successive cycle, the original template strands plus all newly generated strands become template strands for the next round of elongation, leading to exponential (geometric) amplification of the specific DNA target region.

The processes of denaturation, annealing and elongation constitute a single cycle. Multiple cycles are required to amplify the DNA target to millions of copies. The formula used to calculate the number of DNA copies formed after a given number of cycles is 2^n, where n is the number of cycles. Thus, a reaction set for 30 cycles results in 2^{30}, or 1,073,741,824, copies of the original double-stranded DNA target region.

- *Final elongation*: This single step is optional, but is performed at a temperature of 70–74 °C (158–165 °F) (the temperature range required for optimal activity of most polymerases used in PCR) for 5–15 minutes after the last PCR cycle to ensure that any remaining single-stranded DNA is fully elongated.
- *Final hold*: The final step cools the reaction chamber to 4–15 °C (39–59 °F) for an indefinite time, and may be employed for short-term storage of the PCR products.

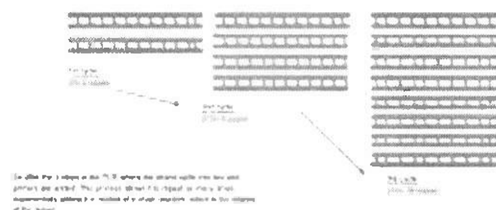


To check whether the PCR successfully generated the anticipated DNA target region (also sometimes referred to as the amplimer or amplicon), agarose gel electrophoresis may be employed for size separation of the PCR products. The size of the PCR products is determined by comparison with a DNA ladder, a molecular weight marker which contains DNA fragments of known sizes, which runs on the gel alongside the PCR products.



1. The DNA double helix is melted apart at T> 90°C and its strands separate.
2. The temperature is decreased to slightly below the T_m of both the primers being used. Both primers bind to the available strands. These primers are supplied in excess to insure that the strands do not only come back and reanneal to one another.
3. Polymerization (extension) occurs via DNA Polymerase in the 5' to 3' direction on each strand.
4. Incorporated additional nucleotides give rise to new strands that extend past the sequence of interest.
5. The previously polymerized strands act as template for the other primer (if forward primer bound first, reverse primer now binds and vice versa).
6. Polymerization occurs via DNA Polymerase in the 5' to 3' direction on each strand, this time ending at the end of the sequence of interest.
7. Incorporated additional nucleotides give rise to new strands that only encode the sequence of interest.
8. The synthesized strands encoding the sequence of interest anneal to one another to form the end product.

Stages



Exponential amplification

As with other chemical reactions, the reaction rate and efficiency of PCR are affected by limiting factors. Thus, the entire PCR process can further be divided into three stages based on reaction progress:

- *Exponential amplification*: At every cycle, the amount of product is doubled (assuming 100% reaction efficiency). After 30 cycles, a single copy of DNA can be increased up to 1,000,000,000 (one billion) copies. In a sense, then, the replication of a discrete strand of DNA is being manipulated in a tube under controlled conditions. The reaction is very sensitive: only minute quantities of DNA must be present.
- *Leveling off stage*: The reaction slows as the DNA polymerase loses activity and as consumption of reagents, such as dNTPs and primers, causes them to become more limited.
- *Plateau*: No more product accumulates due to exhaustion of reagents and enzyme.

Optimization

In practice, PCR can fail for various reasons, such as sensitivity or contamination. **Contamination** with extraneous DNA can lead to spurious products and is addressed with lab protocols and procedures that separate pre-PCR mixtures from potential DNA contaminants. For instance, if DNA from a crime scene is analyzed, a single DNA molecule from lab personnel could be amplified and misguide the investigation. Hence the PCR-setup areas is separated from the analysis or purification of other PCR products, disposable plasticware used, and the work surface between reaction setups needs to be thoroughly cleaned.

Specificity can be adjusted by experimental conditions so that no spurious products are generated. Primer-design techniques are important in improving PCR product yield and in avoiding the formation of unspecific products. The usage of alternate buffer components or polymerase enzymes can help with amplification of long or otherwise problematic regions of DNA. For instance, Q5 polymerase is said to be ~280 times less error-prone than Taq polymerase. Both the running parameters (e.g. temperature and duration of cycles), or the addition of reagents, such as formamide, may increase the specificity and yield of PCR. Computer simulations of theoretical PCR results (Electronic PCR) may be performed to assist in primer design.

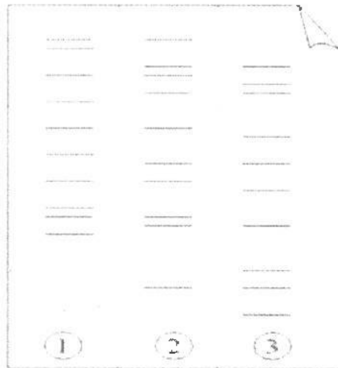
Applications

Selective DNA isolation

PCR allows isolation of DNA fragments from genomic DNA by selective amplification of a specific region of DNA. This use of PCR augments many ways, such as generating hybridization probes for Southern or northern hybridization and DNA cloning, which require larger amounts of DNA, representing a specific DNA region. PCR supplies these techniques with high amounts of pure DNA, enabling analysis of DNA samples even from very small amounts of starting material.

Other applications of PCR include DNA sequencing to determine unknown PCR-amplified sequences in which one of the amplification primers may be used in Sanger sequencing, isolation of a DNA sequence to expedite recombinant DNA technologies involving the insertion of a DNA sequence into a plasmid, phage, or cosmid (depending on size) or the genetic material of another organism. Bacterial colonies *(such as E. coli)* can be rapidly screened by PCR for correct DNA

vector constructs. PCR may also be used for genetic fingerprinting; a forensic technique used to identify a person or organism by comparing experimental DNAs through different PCR-based methods.



Electrophoresis of PCR-amplified DNA fragments:

1. Father
2. Child
3. Mother

The child has inherited some, but not all, of the fingerprints of each of its parents, giving it a new, unique fingerprint.

Some PCR fingerprint methods have high discriminative power and can be used to identify genetic relationships between individuals, such as parent-child or between siblings, and are used in paternity testing (Fig. 4). This technique may also be used to determine evolutionary relationships among organisms when certain molecular clocks are used (i.e. the 16S rRNA and recA genes of microorganisms).

Amplification and quantification of DNA

Because PCR amplifies the regions of DNA that it targets, PCR can be used to analyze extremely small amounts of sample. This is often critical for forensic analysis, when only a trace amount of DNA is available as evidence. PCR may also be used in the analysis of ancient DNA that is tens of thousands of years old. These PCR-based techniques have been successfully used on animals, such as a forty-thousand-year-old mammoth, and also on human DNA, in applications ranging from the analysis of Egyptian mummies to the identification of a Russian tsar and the body of English king Richard III.

Quantitative PCR or Real Time PCR (qPCR, not to be confused with RT-PCR) methods allow the estimation of the amount of a given sequence present in a sample—a technique often applied to quantitatively determine levels of gene expression. Quantitative PCR is an established tool for DNA quantification that measures the accumulation of DNA product after each round of PCR amplification.

qPCR allows the quantification and detection of a specific DNA sequence in real time since it measures concentration while the synthesis process is taking place. There are two methods for

simultaneous detection and quantification. The first method consists of using fluorescent dyes that are retained nonspecifically in between the double strands. The second method involves probes that code for specific sequences and are fluorescently labeled. Detection of DNA using these methods can only be seen after the hybridization of probes with its complementary DNA (cDNA) takes place. An interesting technique combination is real-time PCR and reverse transcription. This sophisticated technique, called RT-qPCR, allows for the quantification of a small quantity of RNA. Through this combined technique, mRNA is converted to cDNA, which is further quantified using qPCR. This technique lowers the possibility of error at the end point of PCR, increasing chances for detection of genes associated with genetic diseases such as cancer. Laboratories use RT-qPCR for the purpose of sensitively measuring gene regulation. The mathematical foundations for the reliable quantification of the PCR and RT-qPCR facilitate the implementation of accurate fitting procedures of experimental data in research, medical, diagnostic and infectious disease applications.

Medical and diagnostic applications

Prospective parents can be tested for being genetic carriers, or their children might be tested for actually being affected by a disease. DNA samples for prenatal testing can be obtained by amniocentesis, chorionic villus sampling, or even by the analysis of rare fetal cells circulating in the mother's bloodstream. PCR analysis is also essential to preimplantation genetic diagnosis, where individual cells of a developing embryo are tested for mutations.

- PCR can also be used as part of a sensitive test for *tissue typing*, vital to organ transplantation. As of 2008, there is even a proposal to replace the traditional antibody-based tests for blood type with PCR-based tests.
- Many forms of cancer involve alterations to *oncogenes*. By using PCR-based tests to study these mutations, therapy regimens can sometimes be individually customized to a patient. PCR permits early diagnosis of malignant diseases such as leukemia and lymphomas, which is currently the highest-developed in cancer research and is already being used routinely. PCR assays can be performed directly on genomic DNA samples to detect translocation-specific malignant cells at a sensitivity that is at least 10,000 fold higher than that of other methods. PCR is very useful in the medical field since it allows for the isolation and amplification of tumor suppressors. Quantitative PCR for example, can be used to quantify and analyze single cells, as well as recognize DNA, mRNA and protein confirmations and combinations.

Infectious disease applications

PCR allows for rapid and highly specific diagnosis of infectious diseases, including those caused by bacteria or viruses. PCR also permits identification of non-cultivatable or slow-growing microorganisms such as mycobacteria, anaerobic bacteria, or viruses from tissue culture assays and animal models. The basis for PCR diagnostic applications in microbiology is the detection of infectious agents and the discrimination of non-pathogenic from pathogenic strains by virtue of specific genes.

Characterization and detection of infectious disease organisms have been revolutionized by PCR in the following ways:

- The *human immunodeficiency virus* (or *HIV*), is a difficult target to find and eradicate. The earliest tests for infection relied on the presence of antibodies to the virus circulating in the bloodstream. However, antibodies don't appear until many weeks after infection, maternal antibodies mask the infection of a newborn, and therapeutic agents to fight the infection don't affect the antibodies. PCR tests have been developed that can detect as little as one viral genome among the DNA of over 50,000 host cells. Infections can be detected earlier, donated blood can be screened directly for the virus, newborns can be immediately tested for infection, and the effects of antiviral treatments can be quantified.
- Some disease organisms, such as that for *tuberculosis*, are difficult to sample from patients and slow to be grown in the laboratory. PCR-based tests have allowed detection of small numbers of disease organisms (both live or dead), in convenient samples. Detailed genetic analysis can also be used to detect antibiotic resistance, allowing immediate and effective therapy. The effects of therapy can also be immediately evaluated.
- The spread of a disease organism through populations of domestic or wild animals can be monitored by PCR testing. In many cases, the appearance of new virulent sub-types can be detected and monitored. The sub-types of an organism that were responsible for earlier epidemics can also be determined by PCR analysis.
- Viral DNA can be detected by PCR. The primers used must be specific to the targeted sequences in the DNA of a virus, and PCR can be used for diagnostic analyses or DNA sequencing of the viral genome. The high sensitivity of PCR permits virus detection soon after infection and even before the onset of disease. Such early detection may give physicians a significant lead time in treatment. The amount of virus ("viral load") in a patient can also be quantified by PCR-based DNA quantitation techniques (see below). A variant of PCR (RT-PCR) is used for detecting viral RNA rather than DNA: in this test the enzyme reverse transcriptase is used to generate a DNA sequence which matches the viral RNA; this DNA is then amplified as per the usual PCR method. RT-PCR is widely used to detect the SARS-CoV-2 viral genome.
- Diseases such as pertussis (or whooping cough) are caused by the bacteria *Bordetella pertussis*. This bacteria is marked by a serious acute respiratory infection that affects various animals and humans and has led to the deaths of many young children. The pertussis toxin is a protein exotoxin that binds to cell receptors by two dimers and reacts with different cell types such as T lymphocytes which play a role in cell immunity. PCR is an important testing tool that can detect sequences within the gene for the pertussis toxin. Because PCR has a high sensitivity for the toxin and a rapid turnaround time, it is very efficient for diagnosing pertussis when compared to culture.

Forensic applications

The development of PCR-based genetic (or DNA) fingerprinting protocols has seen widespread application in forensics:

- DNA samples are often taken at crime scenes and analyzed by PCR.

  In its most discriminating form, *genetic fingerprinting* can uniquely discriminate any one person from the entire population of the world. Minute samples of DNA can be isolated from a crime scene, and compared to that from suspects, or from a DNA database of earlier

evidence or convicts. Simpler versions of these tests are often used to rapidly rule out suspects during a criminal investigation. Evidence from decades-old crimes can be tested, confirming or exonerating the people originally convicted.

- Forensic DNA typing has been an effective way of identifying or exonerating criminal suspects due to analysis of evidence discovered at a crime scene. The human genome has many repetitive regions that can be found within gene sequences or in non-coding regions of the genome. Specifically, up to 40% of human DNA is repetitive. There are two distinct categories for these repetitive, non-coding regions in the genome. The first category is called variable number tandem repeats (VNTR), which are 10–100 base pairs long and the second category is called short tandem repeats (STR) and these consist of repeated 2–10 base pair sections. PCR is used to amplify several well-known VNTRs and STRs using primers that flank each of the repetitive regions. The sizes of the fragments obtained from any individual for each of the STRs will indicate which alleles are present. By analyzing several STRs for an individual, a set of alleles for each person will be found that statistically is likely to be unique. Researchers have identified the complete sequence of the human genome. This sequence can be easily accessed through the NCBI website and is used in many real-life applications. For example, the FBI has compiled a set of DNA marker sites used for identification, and these are called the Combined DNA Index System (CODIS) DNA database. Using this database enables statistical analysis to be used to determine the probability that a DNA sample will match. PCR is a very powerful and significant analytical tool to use for forensic DNA typing because researchers only need a very small amount of the target DNA to be used for analysis. For example, a single human hair with attached hair follicle has enough DNA to conduct the analysis. Similarly, a few sperm, skin samples from under the fingernails, or a small amount of blood can provide enough DNA for conclusive analysis.
- Less discriminating forms of DNA fingerprinting can help in *DNA paternity testing*, where an individual is matched with their close relatives. DNA from unidentified human remains can be tested, and compared with that from possible parents, siblings, or children. Similar testing can be used to confirm the biological parents of an adopted (or kidnapped) child. The actual biological father of a newborn can also be confirmed (or ruled out).
- The PCR AMGX/AMGY design has been shown to not only facilitate in amplifying DNA sequences from a very minuscule amount of genome. However it can also be used for real-time sex determination from forensic bone samples. This provides a powerful and effective way to determine gender in forensic cases and ancient specimens.

Research applications

PCR has been applied to many areas of research in molecular genetics:

- PCR allows rapid production of short pieces of DNA, even when not more than the sequence of the two primers is known. This ability of PCR augments many methods, such as generating *hybridization probes* for Southern or northern blot hybridization. PCR supplies these techniques with large amounts of pure DNA, sometimes as a single strand, enabling analysis even from very small amounts of starting material.

- The task of *DNA sequencing* can also be assisted by PCR. Known segments of DNA can easily be produced from a patient with a genetic disease mutation. Modifications to the amplification technique can extract segments from a completely unknown genome, or can generate just a single strand of an area of interest.
- PCR has numerous applications to the more traditional process of *DNA cloning*. It can extract segments for insertion into a vector from a larger genome, which may be only available in small quantities. Using a single set of 'vector primers', it can also analyze or extract fragments that have already been inserted into vectors. Some alterations to the PCR protocol can *generate mutations* (general or site-directed) of an inserted fragment.
- *Sequence-tagged sites* is a process where PCR is used as an indicator that a particular segment of a genome is present in a particular clone. The Human Genome Project found this application vital to mapping the cosmid clones they were sequencing, and to coordinating the results from different laboratories
- An application of PCR is the phylogenic analysis of DNA from *ancient sources*, such as that found in the recovered bones of Neanderthals, from frozen tissues of mammoths, or from the brain of Egyptian mummies. In some cases the highly degraded DNA from these sources might be reassembled during the early stages of amplification.
- A common application of PCR is the study of patterns of *gene expression*. Tissues (or even individual cells) can be analyzed at different stages to see which genes have become active, or which have been switched off. This application can also use quantitative PCR to quantitate the actual levels of expression
- The ability of PCR to simultaneously amplify several loci from individual sperm[43] has greatly enhanced the more traditional task of *genetic mapping* by studying chromosomal crossovers after meiosis. Rare crossover events between very close loci have been directly observed by analyzing thousands of individual sperms. Similarly, unusual deletions, insertions, translocations, or inversions can be analyzed, all without having to wait (or pay) for the long and laborious processes of fertilization, embryogenesis, etc.
- Site-directed mutagenesis: PCR can be used to create mutant genes with mutations chosen by scientists at will. These mutations can be chosen in order to understand how proteins accomplish their functions, and to change or improve protein function.

Advantages

PCR has a number of advantages. It is fairly simple to understand and to use, and produces results rapidly. The technique is highly sensitive with the potential to produce millions to billions of copies of a specific product for sequencing, cloning, and analysis. qRT-PCR shares the same advantages as the PCR, with an added advantage of quantification of the synthesized product. Therefore, it has its uses to analyze alterations of gene expression levels in tumors, microbes, or other disease states.

PCR is a very powerful and practical research tool. The sequencing of unknown etiologies of many diseases are being figured out by the PCR. The technique can help identify the sequence of previously unknown viruses related to those already known and thus give us a better understanding of the disease itself. If the procedure can be further simplified and sensitive non-radiometric detection systems can be developed, the PCR will assume a prominent place in the clinical laboratory for years to come.

Limitations

One major limitation of PCR is that prior information about the target sequence is necessary in order to generate the primers that will allow its selective amplification. This means that, typically, PCR users must know the precise sequence(s) upstream of the target region on each of the two single-stranded templates in order to ensure that the DNA polymerase properly binds to the primer-template hybrids and subsequently generates the entire target region during DNA synthesis.

Like all enzymes, DNA polymerases are also prone to error, which in turn causes mutations in the PCR fragments that are generated.

Another limitation of PCR is that even the smallest amount of contaminating DNA can be amplified, resulting in misleading or ambiguous results. To minimize the chance of contamination, investigators should reserve separate rooms for reagent preparation, the PCR, and analysis of product. Reagents should be dispensed into single-use aliquots. Pipettors with disposable plungers and extra-long pipette tips should be routinely used. It is moreover recommended to ensure that the lab set-up follows a unidirectional workflow. No materials or reagents used in the PCR and analysis rooms should ever be taken into the PCR preparation room without thorough decontamination.

Environmental samples that contain humic acids may inhibit PCR amplification and lead to inaccurate results.

History

The heat-resistant enzymes that are a key component in polymerase chain reaction were discovered in the 1960s as a product of a microbial life form that lived in the superheated waters of Yellowstone's Mushroom Spring.

A 1971 paper in the *Journal of Molecular Biology* by Kjell Kleppe and co-workers in the laboratory of H. Gobind Khorana first described a method of using an enzymatic assay to replicate a short DNA template with primers *in vitro*.[80] However, this early manifestation of the basic PCR principle did not receive much attention at the time and the invention of the polymerase chain reaction in 1983 is generally credited to Kary Mullis.[81][*page needed*]

"Baby Blue", a 1986 prototype machine for doing PCR

When Mullis developed the PCR in 1983, he was working in Emeryville, California for Cetus Corporation, one of the first biotechnology companies, where he was responsible for synthesizing short chains of DNA. Mullis has written that he conceived the idea for PCR while cruising along the Pacific Coast Highway one night in his car. He was playing in his mind with a

new way of analyzing changes (mutations) in DNA when he realized that he had instead invented a method of amplifying any DNA region through repeated cycles of duplication driven by DNA polymerase. In *Scientific American*, Mullis summarized the procedure: "Beginning with a single molecule of the genetic material DNA, the PCR can generate 100 billion similar molecules in an afternoon. The reaction is easy to execute. It requires no more than a test tube, a few simple reagents, and a source of heat." DNA fingerprinting was first used for paternity testing in 1988.

Mullis has credited his use of LSD as integral to his development of PCR: "Would I have invented PCR if I hadn't taken LSD? I seriously doubt it. I could sit on a DNA molecule and watch the polymers go by. I learnt that partly on psychedelic drugs."

Mullis and biochemist Michael Smith, who had developed other essential ways of manipulating DNA, were jointly awarded the Nobel Prize in Chemistry in 1993, seven years after Mullis and his colleagues at Cetus first put his proposal to practice. Mullis's 1985 paper with R. K. Saiki and H. A. Erlich, "Enzymatic Amplification of β-globin Genomic Sequences and Restriction Site Analysis for Diagnosis of Sickle Cell Anemia"—the polymerase chain reaction invention (PCR)—was honored by a Citation for Chemical Breakthrough Award from the Division of History of Chemistry of the American Chemical Society in 2017.

At the core of the PCR method is the use of a suitable DNA polymerase able to withstand the high temperatures of >90 °C (194 °F) required for separation of the two DNA strands in the DNA double helix after each replication cycle. The DNA polymerases initially employed for in vitro experiments presaging PCR were unable to withstand these high temperatures. So the early procedures for DNA replication were very inefficient and time-consuming, and required large amounts of DNA polymerase and continuous handling throughout the process.

The discovery in 1976 of *Taq polymerase*—a DNA polymerase purified from the thermophilic bacterium, *Thermus aquaticus*, which naturally lives in hot (50 to 80 °C (122 to 176 °F)) environments such as hot springs—paved the way for dramatic improvements of the PCR method. The DNA polymerase isolated from *T. aquaticus* is stable at high temperatures remaining active even after DNA denaturation, thus obviating the need to add new DNA polymerase after each cycle. This allowed an automated thermocycler-based process for DNA amplification.

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A Project Report
On

A STUDY ABOUT COVID 19 SYMPTOMS, CAUSES AND POST COVID COMPLICATIONS

By
Madiha Afreen, II B.Sc. (Bt.B.Z.)
K.Rajeshwari, II B.Sc. (Bt.B.C.)
B.Kalyani, II B.Sc. (Bt.B.C.)
S.Soujanya, II B.Sc. (Bt.B.C.)
Md.Afreena, II B.Sc. (Bt.B.C.)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

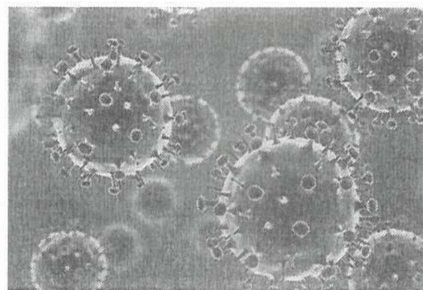
DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN
KARIMNAGAR
(Affiliated to Satavahana University)

A STUDY ABOUT COVID-19 SYMPTOMS, CAUSES AND POST COVID COMPLICATIONS

CORONA VIRUS:

Corona viruses are a group of viruses belonging to the family of Corona viridae, which infect both animals and humans. Human corona viruses can cause mild disease similar to a common cold, while others cause more severe disease (such as MERS - Middle East Respiratory Syndrome and SARS – severe acute respiratory syndrome). A new corona virus that previously has not been identified in humans emerged in Wuhan, China in December 2019. Signs and symptoms include respiratory symptoms and include fever, cough and shortness of breath. In more severe cases, infection can cause pneumonia, severe acute respiratory syndrome and sometimes death. Standard recommendations to prevent the spread of COVID-19 include frequent cleaning of hands using alcohol-based hand rub or soap and water; covering the nose and mouth with a flexed elbow or disposable tissue when coughing and sneezing; and avoiding close contact with anyone that has a fever and cough. WHO is working closely with global experts, governments and partners to rapidly expand scientific knowledge on this new virus and to provide timely advice on measures to protect people's health and prevent the spread of this outbreak?



HOW IT SPREADS:

The virus can spread from an infected person's mouth or nose in small liquid particles when they cough, sneeze, speak, sing or breathe. These particles range from larger respiratory droplets to smaller aerosols.

You can be infected by breathing in the virus if you are near someone who has COVID-19, or by touching a contaminated surface and then your eyes, nose or mouth. The virus spreads more easily indoors and in crowded settings.

- COVID-19 affects different people in different ways. Most infected people will develop mild to moderate illness and recover without hospitalization.

- **Most common symptoms:**
- Fever
- Cough
- Tiredness
- Loss of taste or smell

- **Less common symptoms:**
- Sore throat
- Headache
- Aches and pains
- Diarrhea
- A rash on skin, or discoloration of fingers or toes
- Red or irritated eyes.

- Protect yourself and others around you by knowing the facts and taking appropriate precautions. Follow advice provided by your local health authority.
- Check with your local health authority for the most relevant guidance for your region.

- **To prevent the spread of COVID-19:**
- Maintain a safe distance from others (at least 1 meter), even if they don't appear to be sick.
- Wear a mask in public, especially indoors or when physical distancing is not possible.
- Choose open, well-ventilated spaces over closed ones. Open a window if indoors.
- Clean your hands often. Use soap and water, or an alcohol-based hand rub.
- Get vaccinated when it's your turn. Follow local guidance about vaccination.
- Cover your nose and mouth with your bent elbow or a tissue when you cough or sneeze.
- Stay home if you feel unwell.
  - If you have a fever, cough and difficulty breathing, seek medical attention. Call in advance so your healthcare provider can direct you to the right health facility. This protects you, and prevents the spared of disease.
- Properly fitted masks can help prevent the spread of the virus from the person wearing the mask to others. Masks alone do not protect against COVID-19, and should be combined with physical distancing and hand hygiene. Follow the advice provided by your local health authority.

- **Asymptomatic cases, mild cases of COVID-19:**
- Isolate yourself in a well-ventilated room.
- Use a triple layer medical mask, discard mask after 8 hours of use or earlier if they become wet or visibly soiled. In the event of a caregiver entering the room, both caregiver and patient may consider using N 95 mask.
- Mask should be discarded only after disinfecting it with 1% Sodium Hypochlorite.
- Take rest and drink a lot of fluids to maintain adequate hydration.
- Follow respiratory etiquettes at all times.
- Frequent hand washing with soap and water for at least 40 seconds or clean with alcohol-based sanitizer.
- Don't share personal items with other people in the household.
- Ensure cleaning of surfaces in the room that are touched often (tabletops, doorknobs, handles, etc.) with 1% hypochlorite solution.
- Monitor temperature daily.
- Monitor oxygen saturation with a pulse oximeter daily.
- Connect with the treating physician promptly if any deterioration of symptoms is noticed.

- **Instructions for caregivers:**
    - Mask: The caregiver should wear a triple layer medical mask. N95 mask may be considered when in the same room with the ill person.
    - Hand hygiene: Hand hygiene must be ensured following contact with ill person or patient's immediate environment.
    - Exposure to patient/patient's environment: Avoid direct contact with body fluids of the patient, particularly oral or respiratory secretions. Use disposable gloves while handling the patient. Perform hand hygiene before and after removing gloves.

- The severity of COVID-19 symptoms can range from very mild to severe. Some people may have only a few symptoms. Some people may have no symptoms at all, but can still spread it (asymptomatic transmission). Some people may experience worsened symptoms, such as worsened shortness of breath and pneumonia, about a week after symptoms start.

Some people experience COVID-19 symptoms for more than four weeks after they're diagnosed. These health issues are sometimes called post-COVID-19 conditions. Some children experience multisystem inflammatory syndrome, a syndrome that can affect some organs and tissues, several weeks after having COVID-19. Rarely, some adults experience the syndrome too.

People who are older have a higher risk of serious illness from COVID-19, and the risk increases with age. People who have existing medical conditions also may have a higher risk of serious illness. Certain medical conditions that may increase the risk of serious illness from COVID-19 include:

- Serious heart diseases, such as heart failure, coronary artery disease or cardiomyopathy
- Cancer
- Chronic obstructive pulmonary disease (COPD)
- Type 1 or type 2 diabetes
- Overweight, obesity or severe obesity
- High blood pressure
- Smoking
- Chronic kidney disease
- Sickle cell disease or thalassemia
- Weakened immune system from solid organ transplants or bone marrow transplants
- Pregnancy
- Asthma
- Chronic lung diseases such as cystic fibrosis or pulmonary hypertension
- Liver disease
- Dementia
- Down syndrome
- Weakened immune system from bone marrow transplant, HIV or some medications
- Brain and nervous system conditions, such as strokes
- Substance use disorders

This list is not complete. Other medical conditions may increase your risk of serious illness from COVID-19.

- **When to see a doctor**

If you have COVID-19 signs or symptoms or you've been in contact with someone diagnosed with COVID-19, contact your health care provider right away for medical advice. Your health care provider will likely recommend that you get tested for COVID-19. If you have emergency COVID-19 symptoms, such as trouble breathing, seek care immediately. If you need to go to a hospital, call ahead so that health care providers can take steps to ensure that others aren't exposed.

If you have emergency COVID-19 signs and symptoms, seek care immediately. Emergency signs and symptoms can include:

- Trouble breathing
- Persistent chest pain or pressure
- Inability to stay awake
- New confusion
- Pale, gray or blue-colored skin, lips or nail beds — depending on skin tone.

➢ **Causes:**

Infection with severe acute respiratory syndrome corona virus 2, or SARS-CoV-2, causes corona virus disease 2019 (COVID-19).

The virus that causes COVID-19 spreads easily among people. Data has shown that the COVID-19 virus spreads mainly from person to person among those in close contact. The virus spreads by respiratory droplets released when someone with the virus coughs, sneezes, breathes, sings or talks. These droplets can be inhaled or land in the mouth, nose or eyes of a person nearby.

Sometimes the COVID-19 virus can spread when a person is exposed to very small droplets or aerosols that stay in the air for several minutes or hours - called **airborne transmission**.

The virus can also spread if you touch a surface with the virus on it and then touch your mouth, nose or eyes. But the risk is low.

The COVID-19 virus can spread from someone who is infected but has no symptoms. This is called **asymptomatic transmission**. The COVID-19 virus can also spread from someone who is infected but hasn't developed symptoms yet. This is called **presymptomatic transmission**.

It's possible to get COVID-19 more than once.

When a virus has one or more new mutations it's called a variant of the original virus. The omicron (B.1.1.529) variant spreads more easily than the original virus that causes COVID-19 and the delta variant. However, omicron appears to cause less severe disease. People who are fully vaccinated can get breakthrough infections and spread the virus to others. But the COVID-19 vaccines are effective at preventing severe illness. This variant also reduces the effectiveness of some monoclonal antibody treatments. Omicron has a few major offshoots (sub lineages), including

BA.5 and BA.2.12.1. BA.5 made up about 88% of COVID-19 infections that had genetic sequencing in the U.S. in August, 2022, according to the CDC.

In April, the CDC downgraded the delta variant from a variant of concern to a variant being monitored. This means that the delta variant isn't currently considered a major public health threat in the U.S.

- **Risk factors:**

Risk factors for COVID-19 appear to include:

- Close contact with someone who has COVID-19, especially someone with symptoms
- Being coughed or sneezed on by an infected person
- Being near an infected person when in an indoor space with poor air flow

- **Complications:**

Although most people with COVID-19 have mild to moderate symptoms, the disease can cause severe medical complications and lead to death in some people. Older adults or people with existing medical conditions are at greater risk of becoming seriously ill with COVID-19.

Complications can include:

- Pneumonia and trouble breathing
- Organ failure in several organs
- Heart problems
- A severe lung condition that causes a low amount of oxygen to go through your bloodstream to your organs (acute respiratory distress syndrome)
- Blood clots
- Acute kidney injury
- Additional viral and bacterial infections.

A Project Report
On

ANTIBACTERIAL ACTIVITY OF AZADIRACHTA INDICA

By
R.Manasa, II B.Sc. (Bt.B.C.)
Ch.Supraja, II B.Sc. (Bt.B.C.)
J.Sravya, II B.Sc. (Bt.B.Z.)
Syeda Madihatul Ifra, II B.Sc. (Bt.Z.C.)
B.Sonali Bendre, II B.Sc. (Bt.B.Z.)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

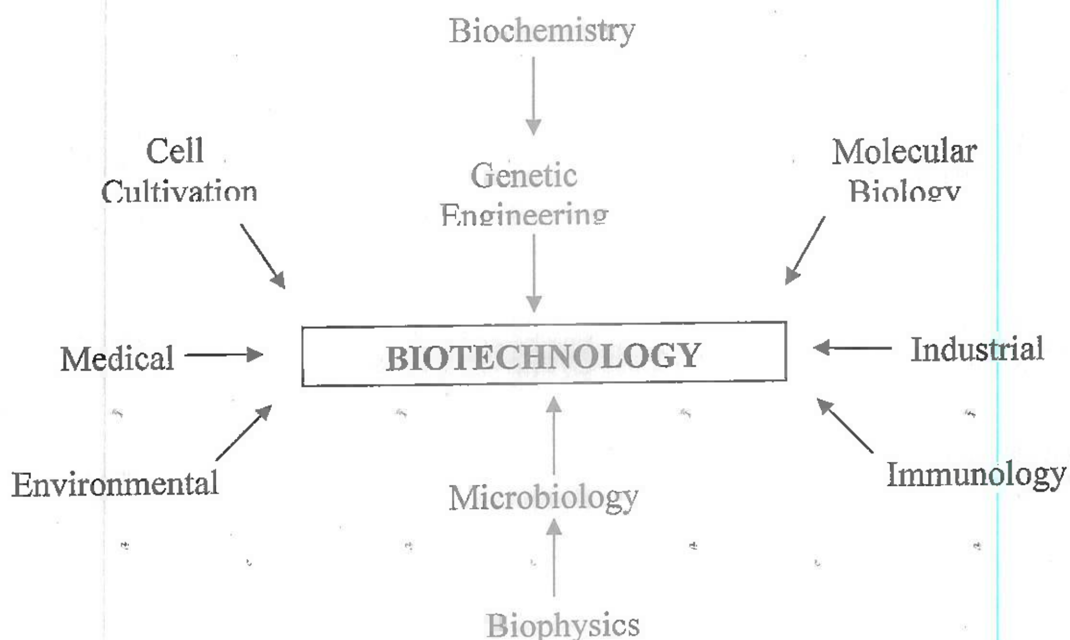
DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN
KARIMNAGAR
(Affiliated to Satavahana University)

INTRODUCTION TO BIOTECHNOLOGY

Biotechnology has been defined as the application of scientific and engineering principles to the processing of materials by biological agents to provide goods and services the town represents the short form of biological technology and is used to describe an interdisciplinary science involving many branches such as genetic engineering biochemistry, biophysics, microbiology, Environmental Engineering and fermentation technology etc.,

The advancements in the areas of modern biology and the emergency of novel technologies especially through recombinant DNA & cell culture techniques have provided new dimensions to several aspects of our life.



Traditional biotechnology can be traced back to the early history – examples include brewing beer, making cheese and using other fermentation processes. Today however most people think of biotechnology as a new science based on a vastly enhanced understanding of the genetic structure of organisms and their modification at the level of cells and molecules. In general terms, the importance of modern biotechnology stems from the fact that it offers the noel possibility of circumventing conventional barriers of genetic incompatibility and sexual hybridization.

AGRICULTURE:-

Biotechnology is making important contributions to agriculture development. Transgenics have already been reported in more than fifty crops. But the new genotypes are yet to be used commercially. Gene manipulation and consequent change in the enzymatic activity is of great significance in agriculture. In this way various techniques are involved, somatic *embryogenetics,* doubled haploid, monochlonar antibody-based diagnostics and Agrobacterium mediated techniques.

MEDICAL AND HUMAN WELFARE:-

With the rapid development and applications of medical biotechnology ranging from simple diagnostic assays to the use of recombinant- DNA technology for production of hormones, vaccines, Enzymes, antibiotics, vitamins and interferon etc., Insulin is used in the treatment of diabetes somatostatin is also an important human growth harmone.

INDUSTRIAL TECHNOLOGY:-

Biotechnology play a vital role in industrial technology. In the Textile industry, analysis isolated from bacteria, fungai, pancreas and malt are used in textile industry as sotening agents for starched clothes. In the leather industry proteolytic enzymes from certain bacteria and fungai are used in the manufacture of leather.

BIOTECHNOLOGY AND PROTEIN ENGINEERING:-

Another very important area of biotechnology is protein Engineering, that will lead to the production of superior Enzymes and storage protein.

FORENSIC MEDICINE :-

DNA finger printing and auto antibody fingerprinting techniques are also proving a great born in forensic medicine for identification of criminals like murderers and rapists through the study of DNA or antibodies from blood and semen stains , urine, tears, saliva, perspiration, or hair roots, etc.,

BIOTECHNOLOGY & ENVIRONMENT:-

Biotechnology is alsobeing used for dealing with environmental problems.

Biotechnological methods have been devised for some environemtntal problems like the following.

1. Pollution control
2. Depletin of natural resources for non-renewable energy
3. Restoratin of degraded lands and
4. Biodiversity conservation. For instance, microbes are being developed to be used as bio-pesticides, biofertilizers biosensors, etc.,

FERMENTATION TECHNOLOGY:-

The fermentation industry is dependent up[on microbial activity for various products such as alcohol's, organic acids, wines etc.,

NEEM

Classification
Botanical name: Azadirachta indica
Family : Maliaceae

The study of crude drugs of plant animal or mineral origin is called pharmocognosy and the study of the action of drugs is called pharmacology.

The curative properties of drugs are due to the presence of complex chemical substances of varied compostion in one or more parts of these plants such as allcaloids, glycosides, Essential oils etc.,

NEEM OR MARGOSA:-

INTRODUCTION: *Azadirachta indica* is distributed throughout India. The tree was worshipped by our ancestors as an invaluable gift of mankind. Every part of the tree is useful to man (may be this reason accounted for the presence of neem tree in every olden home) in the olden days for this reason there were neem trees in every house. It was generally called as the "*Herbal Indian doctor*".

The leaves are reported to be employed in bronchial catarrh, dysentery and diarrhoea. The leaf juice possesses antiseptic, insecticidal and parasiticidal properties. It is used to check haemorrhage from cuts, bruises and wounds. *A.indica* contains proteins carbohydrates, fibre, cao, K_2O, P_2O_5, MgO, b-sitosterol and tannins.

USES:-

Every part of the tree, leaf, flower, fruit seed, wood & roots has been in use especially in the Ayurvedic and unani system of medicine.

1. Decotion of leaves in an effective antidote for round worms. Leaves have medicinal properties like blood purifier carminative and anti-diabetic, they also have Antiseptic and Anti-fungal properties.
2. Recent studies in USA have shown that neem leaf Extracts can inhibits certain Enzymes in the hepatitis-B virus and herpes virus. Germany & U.K are on their way to extract which inhibits the division of AIDS infected cells.
3. The latest reports clearly demonstrate that neem is a powerful immune system, booster specifically enhancing the "killer T" That are the bodies first defence against infection.
4. Seed contain 40-49% oil, neem oil used in making soaps, tooth pastes, herbal shampoos and anti-like shampoos for dogs. It is found helpful in some chronic skin diseases and ulcers. It also cure piles. It has some external application for rheumatism, Leprosy and sprains neem oil is also reported to have Anti-fertility properties
5. Flowers are useful in some cases of dyspepsia and general weakness. Flowers along with leaves are used for dressing of wounds.
6. The bark of the stem and root contain compounds like nimbin and nimbidin. The bark is used in dental diseases like bleeding gums and phorrhoea, jaundice, various

skin diseases like eczema etc., and as an anti-allergic agent bark has also Anti-fungal and protozoan property.

7. Neem seeds powder and leaves provides an excellent bulk to the cattle and animal nutrients processed neem. It is also used for the poultry feed.
8. Derivatives of neem have been reported to acts as insecticide, fungicide & nematicide.
9. Neem is a potential sources of organic manure which is rich in plant nutrients like nitrogen, phospherous and potassium and is anti-microbial. Neem seed powder is used asn an organic fertilizers.
10. As the wood of neem is resistant to pests, it is having some timber values.

ANTIBACTERIAL ACTIVITY ON VARIOUS PARTS OF NEEM

MATERIALS AND METHODS:-

COLLECTION OF MEDICINAL PLANT:- The Medicinal *plant Azadirachta indica* was selected and collected their parts like, root stem and leaves around New science Degree college campus at warangal District.

The collected parts of medicinal plants were brought into the laboratory to determine antibacterial activity.

PREPARATION OF EXTRACTS:-

The collected parts (root, stem and leaf) of medicinal plant were cleaned and dried under shade. The dried plant materials were then ground well to fine powder. Powdered plant materials were successively extracted with alcohol and acetone. The alcohol and acetone extracts, were then filtered and keptin over at 40^0C for 24 hours to evaporate the alcohol and acetone from it. A dark brown and greenish black residues were obtained which are used to determine antibacterial activities.

SELECTION OF MICRO ORGANISMS:-

Enterobacter acrogenes

Bacillus cerens

Proteins

Vulgaris

Pseudomonas aerugenosa

ANTIMICROBIAL TESTING:-

Nutrient agar medium was prepared and sterilized by an autoclave. In an aseptic roam, they poured into sterile petridishes to a uniform, depth of 4mm and then allowed to solidify at room temperature after solidification, the test organism were inoculated with the help of a sterile spreader in bacterial culture or suspension. Thus provided the uniform surface growth of bacterium and is used for antibacterial sensitivity studies.

Then the sterile filter paper discs (6mm) containing samples (100 µl) were immensed in plant extracts and was placed over the solidified agar in such a way that there is no over lapping of zone of inhibitin plates were kept at room temperature for half an hour for the diffusion of the sample in to the agar media. The organism inoculated petridishes were incubated at 37^0C for 48 horus. After the incubating period is over, the zone of inhibition produced by the sample with different organism in different plates were measured and recorded.

OBSERVATION:-

Zone of inhibition is observed in the plates inoculated with Enterobacter aerogenes, Bacillus Cerues, Proteus vulgaris, Pseudomonas aerugenosa.

RESULT:-

From the above experiment it has been concluded that Azadirachta indica has Antibacterial activity.

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A Project Report

On

ANALYSIS OF VARIOUS MILK PHYSICAL AND BIOCHEMICAL PROPERTIES

By

E.Ramya, II B.Sc. (Bt.B.C.)

E.Anjali, II B.Sc. (Bt.B.C.)

A.Anjali, II B.Sc. (Bt.B.C.)

A.Sravani, II B.Sc. (Bt.B.Z.)

K.Ravali, II B.Sc. (Bt.B.Z.)

Guided By

Sri. K.Satheesh Kumar

Lecturer in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN KARIMNAGAR

(Affiliated to Satavahana University)

ANALYSIS OF VARIOUS MILK: PHYSICAL AND BIOCHEMICAL PROPERTIES

INTRODUCTION

Although milk is a liquid and most often considered a drink, it contains between 12 and 13 percent total solids and perhaps should be regarded as a food. In contrast, many "solid" foods, such as tomatoes, carrots, and lettuce, contain as little as 6 percent solids.

Many factors influence the composition of milk, including breed, genetic constitution of the individual cow, age of the cow, stage of lactation, interval between milkings, and certain disease conditions. Since the last milk drawn at each milking is richest in fat, the completeness of milking also influences a sample. In general, the type of feed only slightly affects the composition of milk, but feed of poor quality or insufficient quantity causes both a low yield and a low percentage of total solids. Current feeding programs utilize computer technology to achieve the greatest efficiency from each animal.

COMPOSITION OF MILK

The composition of milk varies among mammals, primarily to meet growth rates of the individual species. The proteins contained within the mother's milk are the major components contributing to the growth rate of the young animals. Human milk is relatively low in both proteins and minerals compared with that of cows and goats.

Goat milk has about the same nutrient composition as cow's milk, but it differs in several characteristics. Goat milk is completely white in colour because all the beta-carotene (ingested from feed) is converted to vitamin A. The fat globules are smaller and therefore remain suspended, so the cream does not rise and mechanical homogenization is unnecessary. Goat milk curd forms into small, light flakes and is more easily digested, much like the curd formed from human milk. It is often prescribed for persons who are allergic to the proteins in cow's milk and for some patients afflicted with stomach ulcers.

Sheep milk is rich in nutrients, having 18 percent total solids (5.8 percent protein and 6.5 percent fat). Reindeer milk has the highest level of nutrients, with 36.7 percent total solids (10.3 percent protein and 22 percent fat). These high-fat, high-protein milks are excellent ingredients for cheese and other manufactured dairy products.

The major components of milk are water, fat, protein, carbohydrate (lactose), and minerals (ash). However, there are numerous other highly important micronutrients such as vitamins, essential amino acids, and trace minerals. Indeed, more than 250 chemical compounds have been identified in milk. The table shows the composition of fresh fluid milk and other dairy products.

Nutrient Composition of Milk (per 100 g)

Type of Milk	Energy (kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Cholesterol (mg)	Vitamin A (IU)	Riboflavin (mg)	Calcium (mg)
*Fortified with vitamin A.									
**Low moisture, part skim.									
Fresh Whole Milk	61	88	3.29	3.34	4.66	14	126	0.162	119
Fresh Low-Fat Milk*	50	89	3.33	1.92	4.80	8	205	0.165	122
Fresh Skim Milk*	35	91	3.41	0.18	4.85	2	204	0.140	123
Evaporated Milk	134	74	6.81	7.56	10.04	29	243	0.316	261
Evaporated Skim Milk*	78	79	7.55	0.20	11.35	4	392	0.309	290
Sweetened Condensed Milk	321	27	7.91	8.70	54.40	34	328	0.416	284
Non fat Dry Milk*	358	4	35.10	0.72	52.19	18	2,370	1.744	1,231

Fat

The fat in milk is secreted by specialized cells in the mammary glands of mammals. It is released as tiny fat globules or droplets, which are stabilized by a phospholipid and protein coat derived from the plasma membrane of the secreting cell. Milk fat is composed mainly of triglycerides—three fatty acid chains attached to a single molecule of glycerol. It contains 65 percent saturated, 32 percent monounsaturated, and 3 percent polyunsaturated fatty acids. The fat droplets carry most of the cholesterol and vitamin A. Therefore, skim milk, which has more than 99.5 percent of the milk fat removed, is significantly lower in cholesterol than whole milk (2 milligrams per 100 grams of milk, compared with 14 milligrams for whole milk) and must be fortified with vitamin A.

Protein

Milk contains a number of different types of proteins, depending on what is required for sustaining the young of the particular species. These proteins increase the nutritional value of milk and other dairy products and provide certain characteristics utilized for many of the processing

methods. A major milk protein is casein, which actually exists as a multisubunit protein complex dispersed throughout the fluid phase of milk. Under certain conditions the casein complexes are disrupted, causing curdling of the milk. Curdling results in the separation of milk proteins into two distinct phases, a solid phase (the curds) and a liquid phase (the whey).

Lactose

Lactose is the principal carbohydrate found in milk. It is a disaccharide composed of one molecule each of the monosaccharides (simple sugars) glucose and galactose. Lactose is an important food source for several types of fermenting bacteria. The bacteria convert the lactose into lactic acid, and this process is the basis for several types of dairy products.

In the diet lactose is broken down into its component glucose and galactose subunits by the enzyme lactase. The glucose and galactose can then be absorbed from the digestive tract for use by the body. Individuals deficient in lactase cannot metabolize lactose, a condition called lactose intolerance. The unmetabolized lactose cannot be absorbed from the digestive tract and therefore builds up, leading to intestinal distress.

Vitamins and minerals

Milk is a good source of many vitamins. However, its vitamin C (ascorbic acid) content is easily destroyed by heating during pasteurization. Vitamin D is formed naturally in milk fat by ultraviolet irradiation but not in sufficient quantities to meet human nutritional needs. Beverage milk is commonly fortified with the fat-soluble vitamins A and D. In the United States the fortification of skim milk and low-fat milk with vitamin A (in water-soluble emulsified preparations) is required by law.

Milk also provides many of the B vitamins. It is an excellent source of riboflavin (B_2) and provides lesser amounts of thiamine (B_1) and niacin. Other B vitamins found in trace amounts are pantothenic acid, folic acid, biotin, pyridoxine (B_6), and vitamin B_{12}.

Milk is also rich in minerals and is an excellent source of calcium and phosphorus. It also contains trace amounts of potassium, chloride, sodium, magnesium, sulfur, copper, iodine, and iron. A lack of adequate iron is said to keep milk from being a complete food.

Physical and biochemical properties

Milk contains many natural enzymes, and other enzymes are produced in milk as a result of bacterial growth. Enzymes are biological catalysts capable of producing chemical changes in organic substances. Enzyme action in milk systems is extremely important for its effect on the flavour and body of different milk products. Lipases (fat-splitting enzymes), oxidases, proteases (protein-splitting enzymes), and amylases (starch-splitting enzymes) are among the more important enzymes that occur naturally in milk. These classes of enzymes are also produced in milk by microbiological action. In addition, the proteolytic enzyme (i.e., protease) rennin, produced in calves' stomachs to coagulate milk protein and aid in nutrient absorption, is used to coagulate milk for manufacturing cheese.

The coagulation of milk is an irreversible change of its protein from a soluble or dispersed state to an agglomerated or precipitated condition. Its appearance may be associated with spoilage, but coagulation is a necessary step in many processing procedures. Milk may be coagulated by rennin or other enzymes, usually in conjunction with heat. Left unrefrigerated, milk may naturally sour or coagulate by the action of lactic acid, which is produced by lactose-fermenting bacteria. This principle is utilized in the manufacture of cottage cheese. When milk is pasteurized and continuously refrigerated for two or three weeks, it may eventually coagulate or spoil owing to the action of psychrophilic or proteolytic organisms that are normally present or result from post pasteurization contamination.

Milk fat is present in milk as an emulsion in a water phase. Finely dispersed fat globules in this emulsion are stabilized by a milk protein membrane, which permits the fat to clump and rise. The rising action is called creaming and is expected in all unhomogenized milk. In the United States, when paper cartons supplanted glass bottles, consumers stopped the practice of skimming cream from the top. Processors then introduced homogenization, a method of preventing gravity separation by forcing milk through very small openings under pressure, thus reducing fat globules to one-tenth their original size. Homogenization is practiced in many dairy processes in order to improve the physical properties of products (*see below* Processing).

Milk and other dairy products are very susceptible to developing off-flavours. Some flavours, given such names as "feed," "barny," or "unclean," are absorbed from the food ingested by the cow and from the odours in its surroundings. Others develop through microbial action due to growth of bacteria in large numbers. Chemical changes can also take place through enzyme action, contact with metals (such as copper), or exposure to sunlight or strong fluorescent light. Quality-control directors are constantly striving to avoid off-flavours in milk and other dairy foods.

A Project Report On

AN OVERVIEW OF SYNTHETIC SEEDS

By
K.Vikasitha, II B.Sc. (Bt.B.C.)
K.Thirumala, II B.Sc. (Bt.B.Z.)
K.Shalini, II B.Sc. (Bt.B.Z.)
L.Srinitha, II B.Sc. (Bt.B.Z.)
R.Sandhya, II B.Sc. (Bt.B.Z.)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN KARIMNAGAR
(Affiliated to Satavahana University)

An Overview of Synthetic Seeds

The decades of research and advancements in biotechnology have presented newer strategies for the improvement of crops and their nutritional value. This includes cereal, legumes, woody forest trees, ornamental plants, etc. However, plant tissue culture, an important component of biotechnology, brought a different era of plant improvement.

The techniques of tissue culture have made it possible to culture and clone plants in labs, conserve specific genes, and allowed for the mass propagation of plants. Among various techniques, somatic embryogenesis gained much popularity because of the formation of an embryo from any somatic cells of the plant.

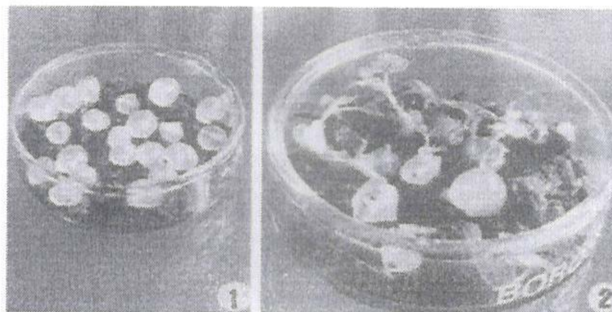
The introduction of somatic embryogenesis increased the demand for artificial seeds and led to the concept of synthetic seeds. It was Murashige who first named the artificial seeds as "synseeds" and he defined it as an "encapsulated single somatic embryo".

However, some plants were recalcitrant to somatic embryogenesis, and that introduced the present idea and definition of synthetic seeds.

What is a Synthetic Seed?

It is defined as encapsulated plant tissues, such as somatic embryos, shoot buds, axillary buds, shoot tips, cell aggregates, and any other micro propagules that have the potential to grow like a plant, under in-vivo or in-vitro conditions when sown as seeds.

Figure: (1) Synseeds or synthetic seeds of a mulberry plant in soil, (2) Germination of synseeds into plantlets.



The two types of synthetic seeds currently being developed include desiccated synthetic seeds and hydrated synthetic seeds.

1. Desiccated Synthetic Seeds

This involves the encapsulation of multiple somatic embryos followed by desiccation. The encapsulating material used in this case is polyoxyethylene (Polyox). This material doesn't allow for the growth of the microorganisms and is non-toxic to embryos.

In this case, a mixture is prepared by using equal volumes of embryo suspension and a 5% (w/v) solution of polyox to give a final concentration of 2.5% polyox. Then by using a pipette the suspension is dispensed as 0.2 ml drops on to Teflon sheets. Then the drops are dried till they themselves leave the Teflon sheet.

2. Hydrated Synthetic Seeds

This involves the encapsulation of a single somatic embryo in hydrogel capsules. This technique is used in those plants which are recalcitrant for the somatic embryogenesis and sensitive to desiccation.

The most popular method of forming hydrated synthetic seeds is using Ca-alginate encapsulation. The procedure followed to produce hydrated synthetic seed involves the mixing of somatic embryos with a 2% (w/v) solution of Na-alginate. Then, the embryo is dropped into a 100 mM solution of Ca(NO3)2 by using a plastic pipette.

Of the two types of synthetic seeds, the desiccated synthetic seeds have greater potential to form synseeds and are closure to the true seeds.

Factors That Affect a Synthetic Seed's Production

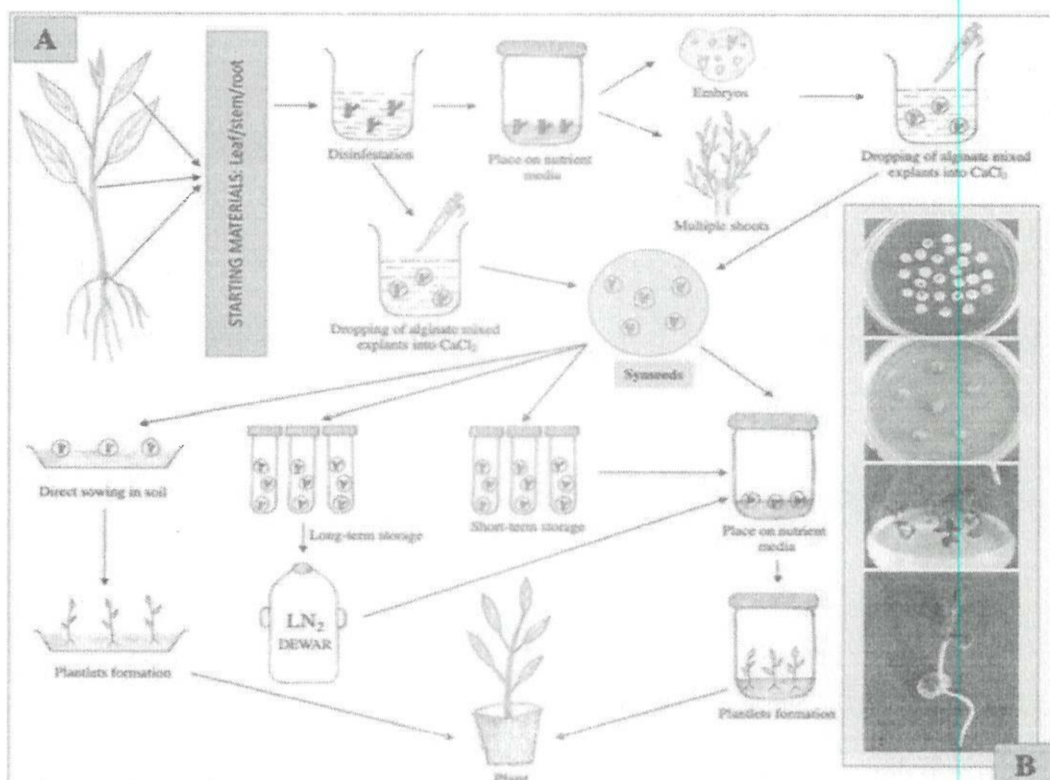
1. Selection of Plant Materials

The synthetic seeds can be produced by using somatic embryos or other plant propagules, However, a specific plant species supports a specific plant material for the production of synthetic seeds.

For example, somatic embryos have been successfully used to produce synseeds in several plant species that include Oryza sativa, Dalbergia sissoo, Curcuma amada, Hemidesmus indicus, etc.

Whereas, some plants support the production of synseeds when nodal segments are used. It includes Eclipta alba, Cannabis sativa, Solanum tuberosum, Gossypium hirsutum, etc.

Figure: (a) Schematic representation of synthetic seed production; (b) synseed seed produced from nodal segments of Tylophora indica.



2. Selection of Encapsulation Matrix

The encapsulation of seeds is an essential factor that determines the production of synseeds. So, there are some qualities that the encapsulating material should have if it's going to be used for the production of synseeds. It includes:

1. It must not damage the embryo.
2. The coating should be mild to protect the embryo.
3. The coating should be durable for rough handling during manufacture, storage, transportation, and planting.
4. The coat must contain all the essential growth materials such as nutrients and growth regulators.
5. The formed synseeds should be transplantable using the existing techniques.

The most widely used encapsulating material is sodium alginate. However, there are some other agents as well that are used with sodium alginate for encapsulation. It includes gelatin, potassium alginate, sodium pectate, and carrageenan.

Advantages of Synthetic seeds Production

The advantages of synthetic seeds production:

1. Easy handling.
2. Short- and long-term storage capacity.
3. Genetic uniformity.
4. Low-cost quality plant materials are required.
5. It allows the transportation and exchange of germplasm between national and international laboratories.

Advantages of synthetic seeds

- This method is for large scale productions.
- It maintains genetic uniformity for a high number of generations. Most plant tissue culture methods fail to maintain genetic uniformity for longer durations.
- According to literature, the costs of producing a plant using this technology is low.
- It facilitates rapid multiplication of plants.
- One of the biggest merit of this method is direct delivery of plant parts (protected with viable coating) to the field.
- These seeds have potential for short and medium term storage without losing viability.
- As compared to plantlets, it is easy to handle and tranport synthetic seeds.

Disadvantages

Though this technology sounds promising, it has some demerits as well. Let us discuss them briefly:

- Somatic embryos have low survival rates for most plant species, which also limits the value of synthetic seeds.
- There are not many protocols available to produce propagules from different plant parts using plant tissue culture methods. Hence less useful material available for producing synthetic seeds.

- In some cases, inefficient maturation of somatic embryos leads to poor germination and hence poor growth and development.
- According to scientists, somatic embryos from some plants species are not capable of germinating out of the capsule or coating. Hence, they are not able to form normal plants rapidly.
- The concentration of coating material is also a limiting factor for producing synthetic seeds. It should have nutrient supplementing materials for facilitating germination and growth.
- When the shape of synthetic seeds is not matching the farm machinery then it is hard to use them for transplantation. Hence, seeds should be transplantable.
- One of the major problems these seeds face is quick drying out of capsules. You need to store them in a humid environment and coat them with hydrophobic materials to prevent drying.

Synthetic seeds have promising applications. There is intensive research and development going on around the world for producing such seeds, especially for important crops. They can be transported easily from one country to another without any quarantine obligations. Hence, these seeds can definitely be an innovative way to increase global food production in the coming years.

Application

1. The production of synthetic seeds facilitates the growth of several plants that have low seed viability, seedless fruit, and poor germination rates, and that depend on mycorrhizal–fungal symbiosis for germination.
2. It is very useful when it comes to genotypes selection, germplasm preservation, and in vitro propagation of endangered, rare, and commercially important plants.
3. It also allows the conservation of plant species through short- and medium-term preservation.

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A Project Report
On

STUDY OF DAIRY PRODUCTS CHEESE AND BUTTER MILK

By

A.Madhumitha, II B.Sc. (Bt.B.C.)
Sumera Khamar, II B.Sc. (Bt.B.C.)
Syeda Habeebunnisa, II B.Sc. (Bt.B.C.)
J.Akanksha, II B.Sc. (Bt.B.C.)
G.Prathyusha, II B.Sc. (Bt.Z.C.)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN
KARIMNAGAR
(Affiliated to Satavahana University)

STUDY OF DAIRY PRODUCTS: CHEESE AND BUTTER MILK

CHEESE

INTRODUCTION

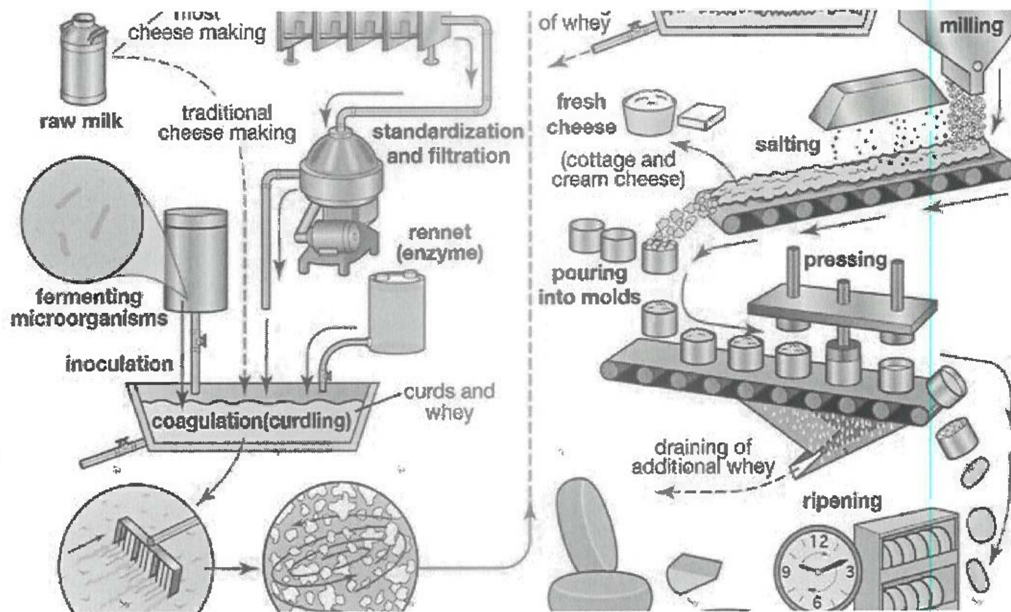
Primitive forms of cheese have been made since humans started domesticating animals. No one knows exactly who made the first cheese, but, according to one ancient legend, it was made accidentally by an Arabian merchant crossing the desert. The merchant put his drinking milk in a bag made from a sheep's stomach. The natural rennin in the lining of the pouch, along with the heat from the sun, caused the milk to coagulate and then separate into curds and whey. At nightfall, the whey satisfied the man's thirst, and the curd (cheese) had a delightful flavour and satisfied his hunger.

From its birthplace in the Middle East, cheese making spread as far as England with the expansion of the Roman Empire. During the Middle Ages, monks and merchants of Europe made cheese an established food of that area. In 1620, cheese and cows were part of the ship's stores carried to North America by the Pilgrims on the *Mayflower*. Until the middle of the 19th century, cheese was a local farm product. Few, if any, distinct varieties of cheese were developed deliberately. Rather, cheese makers in each locality made a cheese that, when ripened under specific conditions of air temperature and humidity, mold, and milk source, acquired certain characteristics of its own. Different varieties appeared largely as a result of accidental changes or modifications in one or more steps of the cheese-making process. Because there was little understanding of the bacteriology and chemistry involved, these changes were little understood and difficult to duplicate. Cheese making was an art, and the process was a closely guarded secret that was passed down from one generation to the next.

With increasing scientific knowledge came a greater understanding of the bacteriological and chemical changes that are necessary to produce many types of cheese. Thus, it has become possible to control more precisely each step in the cheese-making process and to manufacture a more uniform product. Cheese making is now a science as well as an art.

Fundamentals of cheese making

The cheese-making process consists of removing a major part of the water contained in fresh fluid milk while retaining most of the solids. Since storage life increases as water content decreases, cheese making can also be considered a form of food preservation through the process of milk fermentation.



The fermentation of milk into finished cheese requires several essential steps: preparing and inoculating the milk with lactic-acid–producing bacteria, curdling the milk, cutting the curd, shrinking the curd (by cooking), draining or dipping the whey, salting, pressing, and ripening. These steps begin with four basic ingredients: milk, microorganisms, rennet, and salt.

Inoculation and curdling

Milk for cheese making must be of the highest quality. Because the natural microflora present in milk frequently include undesirable types called psychrophiles, good farm sanitation and pasteurization or partial heat treatment are important to the cheese-making process. In addition, the milk must be free of substances that may inhibit the growth of acid-forming bacteria (e.g., antibiotics and sanitizing agents). Milk is often pasteurized to destroy pathogenic microorganisms and to eliminate spoilage and defects induced by bacteria. However, since pasteurization destroys the natural enzymes found in milk, cheese produced from pasteurized milk ripens less rapidly and less extensively than most cheese made from raw or lightly heat-treated milk.

During pasteurization, the milk may be passed through a standardizing separator to adjust the fat-to-protein ratio of the milk. In some cases the cheese yield is improved by concentrating protein in a process known as ultrafiltration. The milk is then inoculated with fermenting microorganisms and rennet, which promote curdling.

The fermenting microorganisms carry out the anaerobic conversion of lactose to lactic acid. The type of organisms used depends on the variety of cheese and on the production process. Rennet is an enzymatic preparation that is usually obtained from the fourth stomach of calves. It contains a number of proteolytic (protein-degrading) enzymes, including rennin and pepsin. Some cheeses, such as cottage cheese and cream cheese, are produced by acid coagulation alone. In the presence of lactic acid, rennet, or both, the milk protein casein clumps together and precipitates out of solution; this is the process known as curdling, or coagulation. Coagulated casein assumes a solid or gellike structure (the curd), which traps most of the fat, bacteria, calcium, phosphate, and other particulates. The remaining liquid (the whey) contains water, proteins resistant to acidic and enzymatic denaturation (e.g., antibodies), carbohydrates (lactose), and minerals.

Lactic acid produced by the starter culture organisms has several functions. It promotes curd formation by rennet (the activity of rennet requires an acidic pH), causes the curd to shrink, enhances whey drainage (syneresis), and helps prevent the growth of undesirable microorganisms during cheese making and ripening. In addition, acid affects the elasticity of the finished curd and promotes fusion of the curd into a solid mass. Enzymes released by the bacterial cells also influence flavour development during ripening.

Salt is usually added to the curd. In addition to enhancing flavour, it helps to withdraw the whey from the curd and inhibits the growth of undesirable microorganisms.

Cutting and shrinking

After the curd is formed, it is cut with fine wire "knives" into small cubes approximately one centimetre (one-half inch) square. The curd is then gently heated, causing it to shrink. The degree of shrinkage determines the moisture content and the final consistency of the cheese. Whey is removed by draining or dipping. The whey may be further processed to make whey cheeses (e.g., ricotta) or beverages, or it may be dried in order to preserve it as a food ingredient.

Ripening

Most cheese is ripened for varying amounts of time in order to bring about the chemical changes necessary for transforming fresh curd into a distinctive aged cheese. These changes are catalyzed by enzymes from three main sources: rennet or other enzyme preparations of animal or vegetable origin added during coagulation, microorganisms that grow within the cheese or on its surface, and the cheese milk itself. The ripening time may be as short as one month, as for Brie, or a year or more, as in the case of sharp cheddar.

The ripening of cheese is influenced by the interaction of bacteria, enzymes, and physical conditions in the curing room. The speed of the reactions is determined by temperature and humidity conditions in the room as well as by the moisture content of the cheese. In most cheeses lactose continues to be fermented to lactic acid and lactates, or it is hydrolyzed to form other sugars. As a result, aged cheeses such as Emmentaler and cheddar have no residual lactose.

In a similar manner, proteins and lipids (fats) are broken down during ripening. The degree of protein decomposition, or proteolysis, affects both the flavour and the consistency of the final cheese. It is especially apparent in Limburger and some blue-mold ripened cheeses. Surface-mold ripened cheeses, such as Brie, rely on enzymes produced by the white *Penicillium camemberti* mold to break down proteins from the outside. When lipids are broken down (as in Parmesan and Romano cheeses), the process is called lipolysis.

The eyes, or holes, typical of Swiss-type cheeses such as Emmentaler and Gruyère come from a secondary fermentation that takes place when, after two weeks, the cheeses are moved from refrigerated curing to a warmer room, where temperatures are in the range of 20 to 24 °C (68 to 75 °F). At this stage, residual lactates provide a suitable medium for propionic acid bacteria (*Propionibacterium shermanii*) to grow and generate carbon dioxide gas. Eye formation takes three to six weeks. Warm-room curing is stopped when the wheels develop a rounded surface and the echo of holes can be heard when the cheese is thumped. The cheese is then moved back to a cold room, where it is aged at about 7 °C (45 °F) for 4 to 12 months in order to develop its typical sweet, nutty flavour.

The unique ripening of blue-veined cheeses comes from the mold spores *Penicillium roqueforti* or *P. glaucum*, which are added to the milk or to the curds before pressing and are activated by air. Air is introduced by "needling" the cheese with a device that punches about 50 small holes into the top. These air passages allow mold spores to grow vegetative cells and spread their greenish blue mycelia, or threadlike structures, through the cheese. *Penicillium* molds are also rich in proteolytic and lipolytic enzymes, so that during ripening a variety of trace compounds also are produced, such as free amines, amino acids, carbonyls, and fatty acids—all of which ultimately affect the flavour and texture of the cheese.

Surface-ripened cheeses such as Gruyère, brick, Port Salut, and Limburger derive their flavour from both internal ripening and the surface environment. For instance, the high-moisture wiping of the surface of Gruyère gives that cheese a fuller flavour than its Emmentaler counterpart. Specific organisms, such as *Brevibacterium linens*, in Limburger cheese result in a reddish brown surface growth and the breakdown of protein to amino nitrogen. The resulting odour is offensive to some, but the flavour and texture of the cheese are pleasing to many.

Not all cheeses are ripened. Cottage, cream, ricotta, and most mozzarella cheeses are ready for sale as soon as they are made. All these cheeses have sweet, delicate flavours and often are combined with other foods.

Varieties of cheese

As a result of the many combinations of milks, cultures, enzymes, molds, and technical processes, literally hundreds of varieties of cheese are made throughout the world. The different types of cheese can be classified in many ways; the most effective is probably according to hardness or ripening method. The table groups several varieties of cheese based on these criteria.

Varieties of cheese, classified by hardness and ripening method

	Ripening method	**Cheese variety**
Very hard	bacteria/enzymes	Asiago, Parmesan, Romano, Sapsago, Sonoma Dry Jack
Hard	bacteria/enzymes	Cantal, cheddar, Colby
	eye-producing bacteria/enzymes	Emmentaler (Swiss), Gruyère, Fontina, Jarlsberg
Semihard/Semisoft	bacteria/enzymes	brick, Edam, Gouda, Monterey Jack, mozzarella, Munster, provolone
	bacteria/enzymes and surface microorganisms	Bel Paese, brick, Limburger, Port Salut, Trappist
	bacteria/enzymes and blue mold	blue, Gorgonzola, Roquefort, Stilton
Soft	bacteria/enzymes and surface microorganisms	Brie, Camembert, Neufchâtel (France), Pont l'Évêque
	unripened	baker's, cottage, cream, feta, Neufchâtel (United States), pot

In recent years different types of cheese have been combined in order to increase variety and consumer interest. For example, soft and mildly flavoured Brie is combined with a more pungent semisoft cheese such as blue or Gorgonzola. The resulting "Blue-Brie" has a bloomy white edible rind,

while its interior is marbled with blue *Penicillium roqueforti* mold. The cheese is marketed under various names such as Bavarian Blue, Cambazola, Lymeswold, and Saga Blue. Another combination cheese is Norwegian Jarlsberg. This cheese results from a marriage of the cultures and manufacturing procedures for Dutch Gouda and Swiss Emmentaler.

Pasteurized process cheese

Some natural cheese is made into process cheese, a product in which complete ripening is halted by heat. The resulting product has an indefinite shelf life. Most process cheese is used in food service outlets and other applications where convenient, uniform melting is required.

Pasteurized process cheese is made by grinding and mixing natural cheese with other ingredients, such as water, emulsifying agents, colouring, fruits, vegetables, or meat. The mixture is then heated to temperatures of 74 °C (165 °F) and stirred into a homogeneous, plastic mass. Process cheese foods, spreads, and products differ from process cheese in that they may contain other ingredients, such as nonfat dry milk, cheese whey, and whey protein concentrates, as well as additional amounts of water.

American cheddar is processed most frequently. However, other cheeses such as washed-curd, Colby, Swiss, Gruyère, and Limburger are similarly processed. In a slight variation, cold pack or club cheese is made by grinding and mixing together one or more varieties of cheese without heat. This cheese food may contain added flavours or ingredients.

Nutrient Composition of Various Cheese types:

Dairy product	Energy (kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Cholesterol (mg)	Vitamin A (IU)	Riboflavin (mg)	Calcium (mg)
Blue cheese	353	42	21.40	28.74	2.34	75	721	0.382	528
Brie cheese	334	48	20.75	27.68	0.45	100	667	0.520	184
Cheddar cheese	403	37	24.90	33.14	1.28	105	1,059	0.375	721
Cottage cheese	103	79	12.49	4.51	2.68	15	163	0.163	60
Cream cheese	349	54	7.55	34.87	2.66	110	1,427	0.197	80
Mozzarella cheese**	280	49	27.47	17.12	3.14	54	628	0.343	731
Parmesan cheese, grated	456	18	41.56	30.02	3.74	79	701	0.386	1,376
Emmentaler (Swiss) eese	376	37	28.43	27.54	3.38	92	845	0.365	961

BUTTERMILK

Because of its name, most people assume buttermilk is high in fat. Actually, the name refers to the fact that buttermilk was once the watery end-product of butter making. Modern buttermilk is made from low-fat or skim milk and has less than 2 percent fat and sometimes none. Its correct name in many jurisdictions is "cultured low-fat milk" or "cultured nonfat milk."

The starting ingredient for buttermilk is skim or low-fat milk. The milk is pasteurized at 82 to 88 °C (180 to 190 °F) for 30 minutes, or at 90 °C (195 °F) for two to three minutes. This heating process is done to destroy all naturally occurring bacteria and to denature the protein in order to minimize wheying off (separation of liquid from solids).

The milk is then cooled to 22 °C (72 °F), and starter cultures of desirable bacteria, such as *Streptococcus lactis, S. cremoris, Leuconostoc citrovorum*, and *L. dextranicum*, are added to develop buttermilk's acidity and unique flavour. These organisms may be used singly or in combination to obtain the desired flavour.

The ripening process takes about 12 to 14 hours (overnight). At the correct stage of acid and flavour, the product is gently stirred to break the curd, and it is cooled to 7.2 °C (45 °F) in order to halt fermentation.

Nutrient Composition of Butter milk:

Dairy Product	Energy (kcal)	Water (g)	Protein (g)	Fat (g)	Carbohydrate (g)	Cholesterol (mg)	Vitamin A (IU)	Riboflavin (mg)	Calcium (mg)
Buttermilk	40	90	3.31	0.88	4.79	4	33	0.154	116

A Project Report
On

STUDY OF DAIRY PRODUCTS BUTTER AND ICE CREAM

By
D.Geethanjali, I B.Sc. (Bt.Z.C.)
M.Sandhya Rani, I B.Sc. (Mb.C.Bt.)
S.Bindhu Reddy, I B.Sc. (Bt.B.C.)
V.Shravani, I B.Sc. (Bt.B.Z.)
Sara Shahewar, I B.Sc. (Bt.B.C.)

Guided By
Sri. K.Satheesh Kumar
Lecturer in Biotechnology

DEPARTMENT OF BIOTECHNOLOGY

GOVERNMENT DEGREE COLLEGE FOR WOMEN KARIMNAGAR
(Affiliated to Satavahana University)

STUDY OF DAIRY PRODUCTS: BUTTER AND ICE CREAM

BUTTER

Composition

Butter is one of the most highly concentrated forms of fluid milk. Twenty litres of whole milk are needed to produce one kilogram of butter. This process leaves approximately 18 litres of skim milk and buttermilk, which at one time were disposed of as animal feed or waste. Today the skim portion has greatly increased in value and is fully utilized in other products.

Commercial butter is 80–82 percent milk fat, 16–17 percent water, and 1–2 percent milk solids other than fat (sometimes referred to as curd). It may contain salt, added directly to the butter in concentrations of 1 to 2 percent. Unsalted butter is often referred to as "sweet" butter. This should not be confused with "sweet cream" butter, which may or may not be salted. Reduced-fat, or "light," butter usually contains about 40 percent milk fat.

Before World War II much of the butter produced in the United States was made from gathered cream. Farmers separated milk on the farm and shipped cans of cream to a butter factory, sometimes once or twice a week. The cream was often sour and needed to be neutralized (with sodium hydroxide) before churning. When transportation and the value of the skim portion improved, whole milk was shipped to the creamery, providing a supply of "sweet cream" (i.e., cream that had not soured) for butter making. With these improvements came the advent of higher-quality butter and the demise of naturally soured buttermilk. Virtually all butter in the United States today is sweet cream butter. A notable exception is butter made from whey cream salvaged in the cheese-making process. The quality of fresh whey cream butter is indistinguishable from sweet cream butter.

Production

Butter is produced when the cream emulsion in unhomogenized milk is destabilized by agitation, or churning. Breaking the emulsion produces butterfat granules the size of rice grains. The granules mat together and separate from the water phase or serum, which is known as buttermilk. (This milky liquid is drained away and is either concentrated or dried, later to become an ingredient in ice cream, candy, or other foods.) The butterfat is then washed with clean water and "worked" (kneaded) until more buttermilk separates and is removed. Ultimately, only about 16 percent of the water and milk solids present in the original milk remains trapped in the butter.

The churning process can take 40 to 60 minutes to complete in a traditional churn, but butter is more commonly made by high-speed continuous "churns" in factories. Although the basic principle is the same, in the continuous churn cream is pumped into a cylinder and mixed by high-speed blades, forming butter granules in seconds. The butter granules are forced through perforated plates while the buttermilk is drained from the system. A salt solution may be added if salted butter is desired. The butter is then worked in a twin screw extruder and emerges ready to be packaged.

Quality concerns

The quality of butter is based on its body, texture, flavour, and appearance. In the United States the Department of Agriculture (USDA) assigns quality grades to butter based on its score on a standard quality point scale. Grade AA is the highest possible grade; Grade AA butter must

achieve a numerical score of 93 out of 100 points based on its aroma, flavour, and texture. Salt (if present) must be completely dissolved and thoroughly distributed. Grade A butter is almost as good, with a score of 92 out of 100 points. Grade B butter is based on a score of 90 points, and it usually is used only for cooking or manufacturing. The flavour of Grade B is not as fresh and sweet, and its body may be crumbly, watery, or sticky.

Additions and treatment

The addition of salt to butter contributes to its flavour and also acts as a preservative. Added in concentrations of approximately 2 percent, all the salt goes into solution in the water phase. Since the water content of butter is less than 16 percent of the total volume, each water droplet can contain more than 10 percent salt. Such a concentration in the water phase limits bacterial growth overall, since the fat portion of butter is generally safe from microbial degradation.

Butter may contain added colouring. Butter from cows that are eating dry, stored feed during the winter may not contain enough beta-carotene for proper colouring, as it does when cows are pasture-fed. In such cases small amounts of a yellow vegetable colouring from the seed of the annatto tree may be added to enhance the colour.

Because butter is so firm when first removed from the refrigerator, it is sometimes whipped to improve spreadability. Generally, volume is increased by 50 percent by whipping in air or, better still, nitrogen or an inert gas in order to prevent oxidation of the fat. Whipped butter, both salted and sweet, is sold in small plastic-coated tubs.

ICE CREAM AND OTHER FROZEN DESSERTS

Ice cream evolved from flavoured ices that were popular with the Roman nobility in the 4th century bce. The emperor Nero is known to have imported snow from the mountains and topped it with fruit juices and honey. In the 13th century Marco Polo was reported to have returned from China with recipes for making water and milk ices.

The discovery that salt would lower the freezing point of cracked ice led to the first practical method of making ice cream. Making ice cream in the home was greatly simplified by the invention of the wooden bucket freezer with rotary paddles. In 1851 the first wholesale ice cream was manufactured in Baltimore. With the development of mechanical refrigeration, widespread distribution of ice cream became possible. Ice cream parlours and drugstore soda counters flourished. With refrigerator-freezers now a standard domestic appliance, more than half of all frozen desserts are consumed at home.

Composition of frozen desserts

Standards for ice cream and most frozen desserts are closely regulated. In the United States, for example, ice cream must contain at least 10 percent fat and 20 percent total milk solids. In freezing, the volume may be doubled by the inclusion of air (known as overrun), but the increase in volume is limited to 100 percent by the requirement that the finished product weigh at least 4.5 pounds per gallon. Total food solids must weigh 1.6 pounds per gallon, thus limiting the water content. Regulations also require all ingredients to be listed, with some additives (such as stabilizers) limited to very small amounts.

The principal frozen desserts are ice cream, frozen custard, ice milk, frozen yogurt, sherbet, and water ices. Ice cream has the highest fat content, ranging from 10 to 20 percent. Frozen custard, or French ice cream, is basically the same formula as ice cream but contains added eggs or egg solids (usually 1.4 percent by weight). Ice milk may be more commonly called "light" or "reduced-fat" ice cream. It contains between 2 and 7 percent fat and at least 11 percent total milk solids. Frozen yogurt is a cultured frozen product containing the same ingredients as ice cream. It must contain at least 3.25 percent milk fat and 8.25 percent milk solids other than fat and must weigh at least five pounds per gallon. Low-fat frozen yogurt contains between 0.5 and 2 percent milk fat. Nonfat frozen yogurt is limited to less than 0.5 percent milk fat. Frozen yogurts should always contain live cultures of bacteria (*see under* Yogurt). The target overrun for ice cream, ice milk, and frozen yogurt is 65 to 100 percent. Premium ice creams may be as low as 20 percent overrun, while soft ice creams are in the 30 to 50 percent range.

Sherbets contain relatively small quantities of milk products. Most standards require between 1 and 2 percent milk fat and between 2 and 5 percent total milk solids. Sherbet contains considerably more sugar and less air than ice cream (the target overrun is 30 to 40 percent), and therefore it is heavier and often contains more calories per serving. Water ices are similar to sherbet, but they contain no milk solids and have a target overrun of 20 to 30 percent.

Imitation ice cream, known as mellorine, is made in some parts of the United States and other countries. It is made with less expensive vegetable oils instead of butterfat but utilizes dairy ingredients for the milk protein part. Mellorines are intended to compete with ice cream in places where butterfat prices are high.

Ice cream manufacture

The essential ingredients in ice cream are milk, cream, sugar, flavouring, and stabilizer. Cheaper ingredients such as dry whey, corn syrup, and artificial flavourings may be substituted to create a lower-cost product.

The first step in ice cream making is formulating a suitable mix. The mix is composed of a combination of dairy ingredients, such as fresh milk and cream, frozen cream, condensed or dried skim, buttermilk, dairy whey, or whey protein concentrate. Sugars may include sucrose, corn syrup, honey, and other syrups. Stabilizers and emulsifiers are added in small amounts to help prevent formation of ice crystals, particularly during temperature fluctuations in storage.

The ice cream mix is pasteurized at no less than 79 °C (175 °F) for 25 seconds. The heated mix is typically homogenized in order to assure a smoother body and texture. Homogenizing also prevents churning (i.e., separating out of fat granules) of the mix in the freezer and increases the viscosity. (Since smaller fat globules have more surface area, the associated milk protein can hydrate more water and produce a more viscous fluid.)

After homogenization, the hot mix is quickly cooled to 4.4 °C (40 °F). The mix must age at this temperature for at least four hours to allow the fat to solidify and fat globules to clump. This aging process results in quicker freezing and a smoother product.

The next step, freezing the mix, is accomplished by one of two methods: continuous freezing, which uses a steady flow of mix, or batch freezing, which makes a single quantity at a time. For both methods, the objective is to freeze the product partially and, at the same time, incorporate air. The freezing process is carried out in a cylindrical barrel that is cooled by a refrigerant, either ammonia or Freon (trademark). The barrel is equipped with stainless steel blades, called dasher blades, which scrape the frozen mixture from the sides of the freezing cylinder and

incorporate or whip air into the product. The amount of air incorporated during freezing is controlled by a pump or the dasher speed. Depending on individual conditions, freezing can be instantaneous in the continuous freezer or require approximately 10 minutes in the batch freezer.

Semifrozen ice cream leaves the freezer at a temperature between −9 and −5 °C (16 and 23 °F). It is placed in a suitable container and conveyed to a blast freezer, where temperatures are in the range of −29 to −34 °C (−20 to −30 °F). While in this room, the ice cream continues to freeze without agitation. Rapid freezing at this stage prevents the formation of large ice crystals and favours a smooth body and texture. The length of time in the hardening room depends on the size of the package, but usually 6 to 12 hours are required to bring the internal ice cream temperature to −18 °C (0 °F) or below. In larger manufacturing plants, final freezing takes place in a hardening tunnel, where packages are automatically conveyed on a continuous belt to the final storage area.

Much of the appeal of ice cream comes from the variety of standard and holiday flavours available throughout the year. Most ice cream manufacturers make a standard mix consisting of milk, cream, sugars, and stabilizers, to which flavours may be added just prior to freezing. High-volume flavours such as vanilla, chocolate, and strawberry may be blended in their own large batch tanks. For flavours with large particles, such as fruit, nuts, cookies, or candy parts, a "feeder" on the outlet of the freezer is used to inject the material. Ingredients such as fruits and nuts are carefully selected and specially treated to avoid introducing unwanted bacteria into the already pasteurized mix.

Ice cream and other frozen desserts require no preservatives and have long shelf lives if they are kept below −23 °C (−10 °F) and are protected from temperature fluctuations. Airtight packaging materials have made it possible to consider frozen storage of six months or longer without loss of flavour or body and texture. When ice cream is finally dipped, composition and overrun will determine ideal scooping temperature. This can vary from −16 to −9 °C (3 to 15 °F), with lower temperatures resulting in less dipping loss but more effort on the part of the server.

Ice cream can also be freeze-dried by the removal of 99 percent of the water. Freeze-drying eliminates the need for refrigeration and provides a high-energy food for hikers and campers and a "filling" centre for candy and other confections.